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Research Article

Microbial Fuel Cell for Dairy Waste Treatment and Electricity Generation

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ABSTRACT

Observing waste as a resource sparks interest in recovering dairy wastewater for resilient sustainability. Dairy wastewater, rich in organic content, is very much suitable for microbial fuel cell (MFC) applications. This paper used MFCs to study electricity generation and dairy waste treatment with paneer whey as a substrate and a biocatalyst culture from dairy development corporation (DDC) sewage targeting for lactose-utilizing strains. Bacteria from DDC sewage found to contain *Klebsiella, Pseudomonas, Salmonella*, and *Escherichia coli,* which were identified by biochemical tests. An yeast *Sungouiella pseudointermedia* was identified molecularly by D1D2 primer amplification and sequencing. A dual-chamber MFC with paneer whey, a bacterial consortium from DDC sewage, and alcohol-treated CNT-coated graphite felt electrodes removed 75.71% of total reducing sugar, 61.03% COD, and 48.91% total phosphorus, generating 25.869 W/m^3. A simpler MFC with paneer whey, a bacterial consortium, and 0.1 M phosphate buffer removed 95.06% of total reducing sugar, 58.27% COD, 65.67% total phosphorus and 67.13% ammoniacal nitrogen but had lower power output. Challenges like pH regulation and equipment limitations are key for optimizing MFC performance and adoption. MFCs show promise as sustainable energy recovery and wastewater treatment solutions.

Keywords: Dairy waste treatment; Electric output; Microbial fuel cell; Bio-electricity; Removal efficiency

Introduction

Waste management is a critical global issue that poses challenges in treatment and disposal, especially with the growth of industries and urbanization. There is growing interest in resource recovery from waste for social and environmental sustainability (Adersa et al., 2021). Dairy industries are significant sources of emerging contaminants, such as estrogens, which enter the environment through wastewater effluents from dairy and livestock activities (Dongre et al., 2021). Dairy wastewater contains high levels of biological oxygen demand (BOD) and chemical oxygen demand (COD), along with fats, lactose, detergents, sanitizing agents, and milk constituents like casein, lactose, fat, and inorganic salts. Industries generate various forms of waste that are often disposed of without treatment (Chaudhary, 2017). The highly biodegradable nature of dairy wastewater necessitates urgent treatment. Common treatment methods for dairy wastewater

include aerobic and anaerobic biological treatments like trickling filters, aerobic lagoons, anaerobic lagoons, sequencing batch reactors (SBR), anaerobic sludge blankets, anaerobic filters, and constructed wetlands. Physical and chemical treatments, such as membrane technology and coagulation/flocculation, are also utilized. However, these conventional techniques have drawbacks such as high costs, energy requirements, and sludge generation. The high energy demands of conventional treatment systems have spurred interest in alternative technologies that are cost-effective and energy-efficient. Microbial fuel cells (MFCs) have emerged as a promising option for wastewater treatment due to their ability to generate electricity from organic waste and renewable biomass (Al-saned et al., 2021). MFCs can efficiently convert this organic matter into electricity, addressing both energy shortages and environmental concerns. In an MFC, bacteria break down organic matter, creating a bio-electrochemical system that produces electricity through microbial metabolism. This process involves the oxidation of organics to generate electrons and protons. Bacteria then transfer electrons to the anode using shuttles or matrices, which flow to the cathode through an external circuit. Protons migrate to the cathode, where they combine with electrons and oxygen to form water. The voltage and current required for electricity production stem from the potential difference between the bacteria's metabolism and the electron acceptor. MFCs not only treat dairy waste but also enhance biodegradation while recovering energy in the form of electricity (Dongre et al., 2021).

There are now numerous private dairies of different sizes in and outside the Kathmandu valley. Despite progress, many dairy industries struggle to meet environmental standards and face pressure from community groups and government regulations. Implementing resource efficient cleaner production (RECP) technique can help these industries reduce their environmental impact, increase productivity, and enhance profitability in the long term (Shrestha, 2017). This research aims to develop a MFC to improve the treatment of industrial dairy waste, specifically Paneer whey, by reducing sugar, COD, phosphorus, and nitrogen content. Additionally, the goal is to generate electricity using substrates from dairy waste, potentially addressing fuel and electricity shortages in the future. Managing dairy waste also benefits in controlling shared air pollution.

Materials and Methods

Sample collection

Freshly prepared paneer whey from the Dairy Development Corporation (DDC) in Lainchaur, Kathmandu, was collected in a sterile bottle and utilized as a substrate in a Microbial Fuel Cell (MFC). Furthermore, sewage from DDC was collected to isolate and identify microbes and to use as a culture in treating the whey in MFC. *Lactobacillus* sp. was collected from Biofuel Laboratory of the Central Department of Biotechnology, Tribhuvan University, Kirtipur, Kathmandu isolated from dairy product. The samples were stored at 4 °C in the Biofuel Laboratory.

Sample analysis

Various physicochemical parameters of paneer whey were analyzed, including chemical oxygen demand (COD), total phosphorus and ammonia-nitrogen using standard method of water analysis (Aniruddha, 2010). Various bacteria were isolated from sewage sample by streaking serially diluted sample to nutrient agar and incubating at 37 °C for 24 hour. To isolate yeast, potato dextrose agar (PDA) plate was used. Different bacteria or yeast were selected according to their morphology and colour. They were repeatedly subcultured to get pure isolates. Biochemical tests were performed. The bacteria were identified by the Bergey's Manual of Systematic Bacteriology (Bergey & Holt, 1994).

Study of lactose utilization and molecular analysis of lactose utilizing yeast isolate

Lactose utilizing yeast isolate was identified by culturing in lactose as sole carbon source which gave growth with bubble formation was molecularly analyzed. Genomic DNA was extracted from the isolate using DNA isolation kit (Promega). PCR was performed (Biorad thermo cycler) using forward primer NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and reverse primer NL-4 (5'-GGTCCGTGTTTCAAGACGG-3') obtained 680 bp fragment. A 25 µl reaction volume with master mix 12.5 µl (2x), $MgCl_2$ 1 µl (25 mM), forward and reverse primer each of 1.5 µl (10 pmol), DNA templete 1 µl (45 ng) nuclease free water (7.5 µl) was used. The PCR conditions were, initial denaturation: 96 °C, 2 min followed by 35 cycles of denaturation: 96 °C, 45 sec; annealing: 52 °C, 45 sec; extension: 72 °C, 2 min; final extension: 72 °C, 10 min and holding at 4 °C (Cocolin et al., 2002). The products were then sequenced in Excelris lab, Ahamdabad, India followed by phylogenetic tree construction using neighbor-joining algorithm in MEGA6.

Microbial fuel cell construction and operation

A dual-chambered microbial fuel cell (MFC) was built with Nafion117 as the Proton Exchange Membrane

(PEM) and graphite felt as both the cathode and anode. The anodic substrate in the MFC was filtered paneer whey supplemented with 5% mixed culture from sewage of DDC or single isolated cultures. A closed circuit was set up using an external 1000 Ω resistor, and the power produced in the MFC was measured with a multi-meter. The MFC was operated for 5-8 days, and samples from the anode were tested daily for physicochemical parameters to assess removal efficiency.

MFC was run with different bacterial and yeast samples in single and mixed cultures, different buffers, chemical oxidizers and modification in electrode coated with CNT composite. The generated power was calculated in terms of Open Circuit Voltage (OCV), Closed Circuit Voltage (CCV), current and power.

Removal of physicochemical parameters after MFC operation

Before starting the MFC operation, the physicochemical parameters of the anodic substrate were measured. After each MFC operation with various catholyte and electrode modifications measurements of total reducing sugar, COD, total phosphorus, and Ammoniacal nitrogen were conducted as stated in Aniruddha, P., 2010. The removal efficiency was calculated by determining the difference between the initial and final concentrations of these parameters and using the formula:

$$\text{Removal efficiency (\%)} = \frac{(\text{Final Conc.} - \text{Initial Conc.})}{\text{Initial Conc.}} \times 100$$

Cyclic voltammetry (CV)

The Hokuto-Denko HA151 Potentiostat (Central Department of Chemistry, Tribhuvan University, Nepal) was utilized in conjunction with the workstation for CV measurements, which were conducted in a three-electrode configuration. A platinum electrode served as the counter electrode, and it was pretreated by washing with distilled water. The reference electrode used was calomel (mercury chloride). Measurements were carried out within the range of -1 V to +1 V. Characterization through cyclic voltammetry (CV) was performed at a scan rate of 0.1 V/sec, with data collected at 10 mV intervals to achieve stable current values. CV was repeated for up to 10 cycles (Molina et al., 2011).

Results and Discussion

Sample analysis and characterization of isolated microbes

The physicochemical analysis of paneer whey included testing for six parameters: pH, temperature, total COD, total phosphorus, and ammoniacal nitrogen using standard method of water analysis (Aniruddha, 2010). They were found to be 5.37, 75 °C, 6891.6 mg/l, 0.23 mg/l and 0.03 mg/l respectively. The biological analysis of the wastewater sample focused on isolating and identifying bacteria biochemically or molecularly. Table 1 showed that paneer whey was found to be of acidic nature which may be primarily caused by the presence of lactic acid bacteria, and the acidic agents used to coagulate milk also contribute to this acidity. Since the whey was freshly collected, the recorded temperature was 75 °C, because during the paneer making process, milk is heated and whey being a liquid retains some heat. Simultaneously, with reference of COD value obtained, the whey was organically rich. However, whey has the potential to be utilized in various fields. According to Patowary et al. (2016), paneer whey with a pH of 5.4 ± 0.3 and rich in COD of upto 65000 ± 3.54 mg/l as per concentration strategy adopted which when discharged to water bodies, it increases the nutrition, that can lead to eutrophication. The phosphorus and ammoniacal nitrogen found in the whey may be due to microbial metabolism of organic compound and acidic pH also contribute to its release. The purpose of determining the parameters was to assess the organic content and nutrient availability in the substrate for use in microbial fuel cells to support the metabolic needs of the microbial community.

Table 1: Biochemical identification of bacteria isolated from DDC sewage.

Tests	*Klebsiella* sp.	*Salmonella* sp.	*Pseudomonas* sp.	*Escherichia* sp.
Gram reaction	Gram negative	Gram negative	Gram negative	Gram negative
Indole test	Negative	Negative	Positive	Positive
Methyl red test	Negative	Positive	Negative	Positive
Voges -Proskaur test	Positive	Negative	Negative	Negative
Citrate test	Positive	Positive	Positive	Negative
Catalase test	Positive	Positive	Positive	Positive
Nitrate test	Negative	Positive	Positive	Positive
Urease test	Positive	Negative	Negative	Negative
Motility test	Non-motile	Motile	Motile	Motile

Upon the bacterial isolation from DDC sewage, four bacteria were biochemically identified of the genera *Klebsiella* sp., *Pseudomonas* sp., *Salmonella* sp. and *Escherichia* sp. (Table 1). The bacteria such as *E. coli, Klebsiella* sp., and *Pseudomonas* sp. are frequently present in wastewater, as reported by Chahal et al. (2016). The purpose of using sewage for bacterial source was because the diverse microbial community in wastewater enhances substrate utilization, maximizes electron donor availability for electricity generation, and promotes synergistic interactions among microbes, ultimately improving the overall performance of microbial fuel cells (MFCs). Furthermore, these bacteria have the ability to adapt to changing environments, making them advantageous for MFC modifications.

In the study by Gunawardena et al. (2008), yeast such as *Saccharomyces cerevisiae* were utilized as a biocatalyst in a glucose-powered microbial fuel cell (MFC), achieving a maximum power output of 146.71 ± 7.7 mW/m^3. So, whether or not the yeast performs well as a biocatalyst, in our research, we isolated and tested the isolated yeast for its ability to ferment lactose. Among the isolated yeast strains, one yeast is found capable to utilize lactose as it showed growth and bubble formation in the media with lactose as sole carbon source (Figure 1) which was molecularly identified as *Sungouiella pseudointermedia* (Figure 2).

Figure 1: Analysis of yeast for lactose utilization; Control (left), Yeast sample (right).

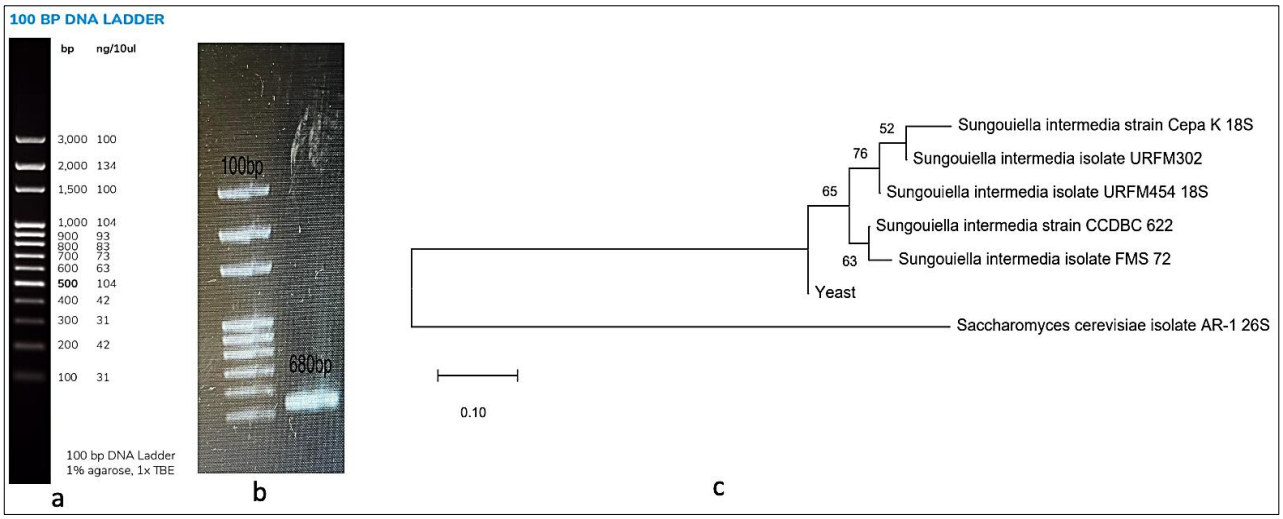

Figure 2: Molecular analysis of yeast by 16S rRNA region amplification; a: marker image, b: gel picture (lane 1:100 bp marker, lane 2: 680 bp PCR product) and c:Phylogenetic tree construct using MEGA 12.

MFC performance in terms of electric output and removal efficiency

The modification in the biocatalyst microbes used, catholyte, and electrode resulted in the optimal configuration for the operation of a microbial fuel cell (MFC) with paneer whey as the anolyte (Table 2). MFCs with mixed bacteria/yeast consistently showed better power output than those with single bacteria due to synergistic effects between species (Han et al., 2024). This was also supported by the study of Ren et al. (2021), where a mixed culture of *Saccharomyces cerevisiae* and *Bacillus subtilis* generated more power than when operated alone. A mixed consortium indeed generated a higher power output than an MFC operated with a single microbe. Despite the mixed consortium generating high power, the power output was overshadowed when the bacterial/yeast consortium was mixed with *Lactobacillus* sp. This may be due to the synergy of *Lactobacillus* sp. with the mixed bacterial/yeast consortium, and *Lactobacillus* sp. which degraded lactose present in paneer whey.

Mixed bacterial/yeast consortium of DDC waste with catholyte 0.1 M phosphate buffer, 0.1 M potassium ferricyanide, and the electrode graphite felt coated with CNT in ethanol (Table 2) generated maximum power of 25.869 mW/m^3. According to Gunawardena et al. (2008), addition of electron mediators like methylene blue and ferricyanide in *Saccharomyces cerevisiae*-based fuel cell showed improved performance, resulting in a maximum power generation of 0.1467 W/m^3 and a maximum open circuit voltage (OCV) of 383.6 mV under 1 KΩ resistance.

The electron acceptor used in the catholyte was potassium ferricyanide, and the buffering solutions were phosphate buffer and sodium acetate. Phosphate buffer helps maintain a suitable pH for electricity-generating bacteria and increases solution conductivity. According to Guerrero et al. (2010), potassium ferricyanide has the potential for a maximum OCV of 710 mV with a maximum power density of 0.92 mW/m^3 using a resistor of 5 KΩ when anaerobic sludge is used. However, in our study, the electric output was comparatively low, which may be due to various influencing factors such as the microbial strain used, their source, external resistor, physicochemical parameters, operating conditions, etc. Graphite felt coated with absolute alcohol-treated CNT stands out among other electrode modifications in terms of power generation. CNT, due to its high surface volume-to-volume ratio, has high electrical conductivity and offers a promising future for improved electrode-microbial interaction. The alcohol treatment helps disperse CNTs evenly, improves their adhesion to the electrode surface, and reduces the internal resistance of the MFC (Basheer et al., 2019).

According to Erbay et al. (2015), microbial growth over the CNT results in excellent charge transfer characteristics due to π-π stacking between the carbon atoms of the graphite and the pili of microbes. This could be the reason behind the maximum power output.

Table 3 revealed the removal efficiencies of MFC at different conditions of operation. Removal efficiencies were found not corelated with electrical performance. This may be due to high lactose utilizing properties of isolated yeast with no recorded electrical performance.

The cyclic voltammogram (CV) exhibited 3 anodic peaks (Figure 3), with two peaks observed between 0.3 V and 0.1 V, likely corresponding to metal oxides in the graphite electrodes. The third peak at 0.5 V indicated the redox peak of electroactive oxide/hydroxide in the carbon electrode. The corresponding cathodic peak was observed at 0.1 V to -0.5 V. The separation between the anodic and cathodic peaks suggested a quasi-reversible system, possibly due to the presence of metal oxide. No distinct cathodic peak was observed for the metal oxide. In the first cycle, the anodic current was influenced by surface activation above 0.2 V. Subsequent cycles showed minimal differences between the fourth and tenth cycles compared to the first cycle, indicating electrode degradation with cycle number, although the degradation was not significant.

Conclusion

In summary, this research underscores the remarkable potential of two-chamber electron-mediated Microbial Fuel Cells (MFCs) utilizing graphite felt electrodes and paneer whey substrate for both bioelectricity generation and dairy waste treatment. The optimized MFC configuration, featuring paneer whey as the anolyte, 0.1 M phosphate buffer with potassium ferricyanide as the cathodic solution, and a specialized bacterial consortium, showcased outstanding performance metrics, including substantial reductions in various pollutants and an impressive maximum power output of 25.869 mW/m^3. Moreover, while simpler MFC setups exhibited effective pollutant removal capabilities, they yielded comparatively lower power outputs. Notably, our findings highlight the superiority of mixed bacterial/yeast cultures over single-strain counterparts, emphasizing the importance of optimizing buffer composition and electron acceptors for enhanced power generation.

Additionally, the promising results obtained with electrodes coated with CNT composite suggest exciting possibilities for further improving power generation efficiency. Addressing challenges such as pH maintenance and equipment limitations will be crucial for maximizing MFC performance and facilitating broader adoption. With continued investment and refinement, MFCs hold significant promise as both sustainable energy recovery solutions and efficient wastewater treatment methods, contributing to a more environmentally sustainable future.

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Table 2: Electric output of MFC with different configurations.

MFC Configuration **Anolyte pH: 5.4** **Catholyte pH: 7.6** **Operating temperature: Room temperature (approx. 25°C)**		**Maximum OCV (mV)**	**Maximum CCV (mV)** (External resistor: 1000 Ω)	**Maximum Current (mA)**	**Maximum Power (mW/m³)**
Anolyte: Paneer whey Catholyte: Phosphate Buffer Electrode: Graphite felt	Biocatalyst *Mixed Bacterial /yeast Consortium of DDC waste* *Lactobacillus spp*. only *Klebsiella spp.* only Mixed Bacterial/yeast consortium of DDC waste + *Lactobacillus spp.*	394.4 390.7 509.4 528.4	157.1	0.060	13.510
Anolyte: Paneer whey Electrode: Graphite Felt Biocatalyst: Mixed bacterial/yeast consortium of DDC waste + *Lactobacillus spp.*	Catholyte 0.1M Sodium acetate Phosphate buffer 0.1M Phosphate buffer + 0.1M Potassium ferricyanide 0.1M Sodium acetate + 0.1M Potassium ferricyanide	429.4 478.2 609.5 515.4	191.2	0.0815	22.261
Anolyte: Paneer whey Biocatalyst: Mixed bacterial/yeast consortium of DDC waste+ *Lactobacillus spp.* Catholyte: 0.1M Phosphate buffer + 0.1M Potassium ferricyanide	Electrode CNT + Ethanol	633	203.7	0.0889	25.869

Table 3: Performance of MFCs in terms of removal efficiency with various configurations.

MFC configuration: Dual Chambered **Anolyte pH: 5.4** **Catholyte pH: 7.6** **Operating temperature: Room temperature (approx. 25°C)**		**Removal (%)**		
		COD	Total Phosphorus	Ammoniacal Nitrogen
Anolyte: Paneer whey Catholyte: 0.1 M Phosphate Buffer Electrode: Graphite felt	Biocatalyst Mixed bacterial/yeast Consortium of DDC waste *Lactobacillus* *Klebsiella* Mixed bacterial/yeast consortium of DDC waste + *Lactobacillus spp.*	50 41.72 60.68 58.27	54.20 36.58 49.88 65.67	63.50 67.13 33.98 67.13
Anolyte: Paneer whey Electrode: Graphite felt Biocatalyst: Mixed bacterial/yeast consortium of DDC waste + *Lactobacillus spp.*	Catholyte 0.1M Sodium acetate 0.1M Phosphate buffer + 0.1M Potassium ferricyanide 0.1M Sodium acetate + 0.1M Potassium ferricyanide	65.51 55.17 53.79	60.38 58.59 53.30	- - -
Anolyte: Paneer whey Biocatalyst: Mixed bacterial consortium of DDC waste + *Lactobacillus spp.* Catholyte: 0.1M Phosphate buffer + 0.1M Potassium ferricyanide	Electrode CNT treated with Ethanol	61.03	48.91	- - -

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